INTRACELLULAR LOCALIZATION OF YEAST (*SACCHAROMYCES CEREVISIAE*) LYSYL – tRNA SYNTHETASE

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Received 29 December 1976
Revised version received 20 March 1977

1. Introduction

The aminoacyl-tRNA synthetases have generally been considered to be soluble components of the protein synthesis system. In the procaryots they seem to be localized in the cytoplasm [1,2].

However, eucaryotic aminoacyl-tRNA synthetases are not found integrally in the soluble fraction of the cytoplasm [2]; they may be associated with ribosomes [3,4], mitochondria or chloroplasts [5].

In certain cases, these enzymes have been found to be bound either to lipids [6], probably derived from cell membranes, or to elongation factors of protein synthesis [7].

In other words, in eucaryotic cells, the aminoacyl-tRNA synthetases are associated with different elements of the protein biosynthetic system. In fact, the efficiency of protein biosynthesis would be favoured by such a regroupment of the different functional elements.

2. Materials and methods

2.1. *Preparation of the enzyme extract*

The lysyl-tRNA synthetase is isolated from the cells of *Saccharomyces cerevisiae* (strain H4), cultured anaerobically at 28°C in a medium containing 30 g/litre glucose and 5 g/litre yeast extract [8]. The cells are gathered during their exponential phase of growth and are transformed into spheroblasts by the following method [9]:

(i) 50 g of fresh yeast (wet weight) are suspended in 150 ml 1.4 M sorbitol buffer: 0.1 M phosphate-citrate (pH 5.8), 0.05 M EDTA, 1.4 M sorbitol, 10 mM mercaptoethanol. This suspension is then exposed to the action of 7 ml of snail extract (suc d'escargot - Industrie Biologique Française) at a temperature of 32°C for 2–3 h.

(ii) Following this treatment, 90% of the cells have been transformed into spheroblasts by the loss of their external wall.

(iii) The spheroblasts are collected by centrifugation for 15 min at 4°C and 3600 X *g*. The cells are then once more suspended in 150 ml 1.4 M sorbitol buffer and centrifuged again in the same manner. This operation is performed twice. The spheroblasts are then exposed to the following osmotic shock: 0.4 M sorbitol, 1 mM EDTA, 0.05 M Tris (pH 7.2), 2 mM phenyl–methyl–sulfonium fluoride (PMSF is a protease inhibitor).

(iv) The lysis is completed by several passages through a potter homogenizer with a teflon piston rotating at 1000 rev/min.

(v) The enzyme extract obtained is centrifuged for 30 min at 15 000 X *g* to eliminate unbroken cells and cellular debris.

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2.2. *Preparation of ribosomes*

Yeast cells are collected and lysed by the same method used in the preparation of the enzyme extract. After centrifugation for 30 min at 15 000 × *g*, aliquots of 14 ml supernatant are layered on to a discontinuous sucrose gradient consisting of 10 ml 2 M sucrose overlayered with an equal volume of 0.7 M sucrose. After 24 h of centrifugation at 22 500 rev/min (SW 25 rotor of a Spinco L2), the polysomes are obtained as a clear and transparent precipitate [10]. The ribosomes are then prepared using the method of Falvey and Staehelin [11]. They are then freed of the initiation and elongation factors by washing with 0.5 M NH_4Cl.

2.3. *Determination of lysyl-tRNA synthetase activity*

The aminoacylation of tRNA is effectuated at 30°C in the following incubation medium: 100 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 2 mM ATP, 0.4 mg total yeast tRNA (Boehringer), 10 μM [^{14}C]lysine (spec. act. 187 Ci/mol) and a given quantity of the enzyme extract. Charged tRNA is measured as previously described [12].

2.4. *Localization of the lysyl-tRNA synthetase*

The localization is effectuated in two ways:

2.4.1. Centrifugation in a discontinuous gradient of 2 M and 1 M sucrose [13]

The enzyme extract is layered on a discontinuous gradient formed by a bottom layer of 2 M sucrose and a top of an equal volume of 1 M sucrose. This is centrifuged in the SW 25 rotor of a Spinco L2 at 22 500 rev/min for 16 h. The fractions are collected and the activity is determined for the formation of lysyl-tRNA.

2.4.2. Column chromatography on 150 M agarose

A 2 × 14.5 cm column is equilibrated with the buffer: 0.05 M Tris–HCl (pH 7.6), 1 mM EDTA, 3 mM $MgCl_2$, 0.5 M dithiothreitol and 0.2 mM PMSF. On the column are layered 2 ml enzyme extract obtained as described above and 20 μl of β-galactosidase. The column is then eluted with the same buffer; 0.7 ml fractions are collected and assayed for the formation of lysyl-tRNA and for the activity of the β-galactosidase. As further markers, the column is then loaded with 1.5 mg yeast ribosomes and 100 μl tritiated water (spec. act. 1.3 × 10^6 dpm/ml). These are eluted under identical conditions.

Measurement of column markers.

(i) Determination of β-galactosidase [14].
The incubation medium contains: 0.1 M Tris (pH 7), 0.1 M NaCl, 0.14 M β-mercaptoethanol, 0.7 g/litre *O*-nitro-phenol-β-D-galacto-pyranoside (ONPG). The enzyme extract is added to 1.5 ml this medium and incubated at 28°C. The reaction is stopped by the addition of 1.5 ml 1 M Na_2CO_3 and the absorption is examined at 420 nm.

(ii) Detection of tritiated water
The radioactivity of the fractions collected from the agarose column is counted with 5 ml Bray's scintillation fluid.

(iii) Detection of the ribosomes
The ribosomes are localized in the fractions eluted from the agarose column by their absorption at 260 nm.

3. Results and discussion

We have previously demonstrated [15] that the native form of lysyl-tRNA synthetase (mol. wt 140 000 d) is bound to a lipid which is indispensable for its activity, and that this native enzyme forms high molecular weight aggregates (greater than 16 S).

It has also been shown [16] by electron microscopy, that the hepatic amino acyl-tRNA synthetases of the rat are found in the form of circular or rectangular particles of about 11–14 nm.

In this study, we have examined the state of the particles to which the enzyme is bound in the yeast cell homogenate.

The use of two very different methods: centrifugation in discontinuous gradients of sucrose (2 M and 1 M) and 150 M agarose column chromatography have lead to analogous results.

By the centrifugation method, one finds that the enzymatic activity is associated with two particulate fractions: the major fraction (67%) is found at the surface of the 1 M cushion of sucrose, the level at which one usually finds cytoplasmic membrane particles. Another smaller fraction of activity (32%), is found at the 1 M:2 M sucrose interface, a zone which contains the endoplasmic reticulum membranes (smooth and rough), as well as the 80 S ribosomes (fig.1) [13].

By the chromatographic method, we found two

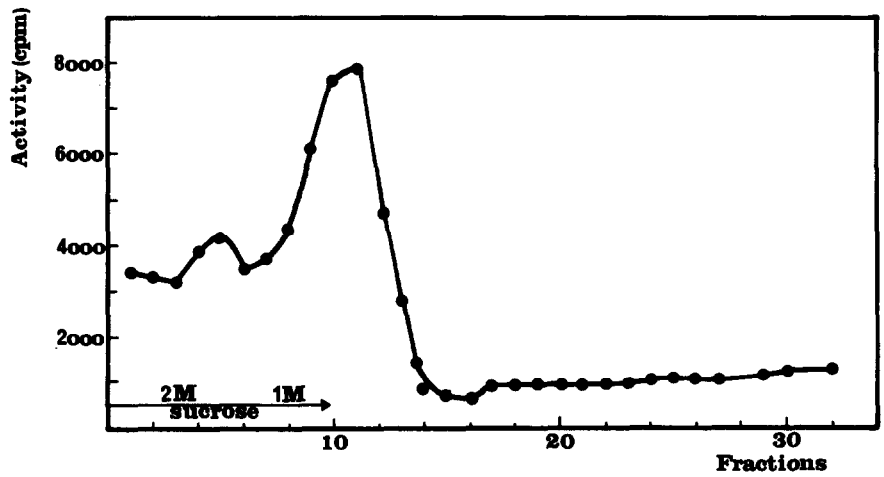

Fig.1. Activity of the lysyl-tRNA synthetase fractionated by centrifugation in a discontinuous gradient of sucrose. The discontinuous gradient is formed by 16.5 ml 2 M sucrose in a bottom layer and an equal volume of 1 M sucrose as top layer. The enzyme extract (0.2 ml) is layered on the surface of the 1 M sucrose. The centrifugation is performed in a SW 25 rotor of a Spinco L2 at 22 500 rev/min for 16 h. The fractions are collected and the activity of the lysyl-tRNA synthetase is determined as described in the Materials and methods section.

peaks of enzymatic activity: a major fraction (62%) being situated immediately at the break through of the column and corresponding to the binding of the enzyme with the membrane fragments; this is revealed by refractometric studies at 600 nm. The enzyme activity peak and the membrane peak are perfectly superimposable. The second activity fraction (38%) is situated just before the elution level of the β-galactosidase and most probably, corresponds to the liaison of the enzyme to the microsomal particles (fig.2).

It is important to emphasize that the use of a gentle technique for the preparation of the enzyme extract diminishes the possibility of an alteration of the enzyme complex [17].

With the technique used for the preparation of the enzyme, one does not obtain free enzymatic activity in the cyto-soluble fraction.

The two methods employed enabled us to identify two forms of the enzyme. A major form, bound to cytoplasmic membranes. A second, minor fraction is composed of high molecular weight aggregates (> 550 000). This enzymatic fraction is probably bound to microsomal particles.

The association between the amino-acyl-tRNA synthetases and cellular particles have been demonstrated by several authors. Tscherne et al. [3], have found such enzymes (extracted from rat liver homogenate) bound to both ribosomes and rough and smooth endoplasmic reticulum membranes. However, Deutscher et al. [18] have demonstrated that most of the aminoacyl-tRNA synthetases (rat hepatocyte) are found in a complex not bound to ribosomes, possessing a molecular weight above one million. Roberts et al. [17] have found the synthetase activity in Erlich ascites cells bound to the ribosomal fraction as well as in the form of a complex with a sedimentation coefficient of 25 S.

These results, apparently contradictory, are in fact concordant. Tscherne et al. [3] and Roberts et al. [17] have shown that following the homogenization of the cells and the isolation of the particular fractions, the eucaryotic aminoacyl-tRNA synthetases may be found in particulate complexes which may or may not be bound to ribosomes.

Our results, obtained with yeast (*Saccharomyces cerevisiae*) enzyme extracts, isolated by gentle techniques, agree with the results obtained with mammalian aminoacyl-tRNA synthetases. The question still remains as to whether these particulate enzyme complexes exist in vivo or are in fact artefactual, being produced at the time of cell lysis.

However, the discovery of a large number of

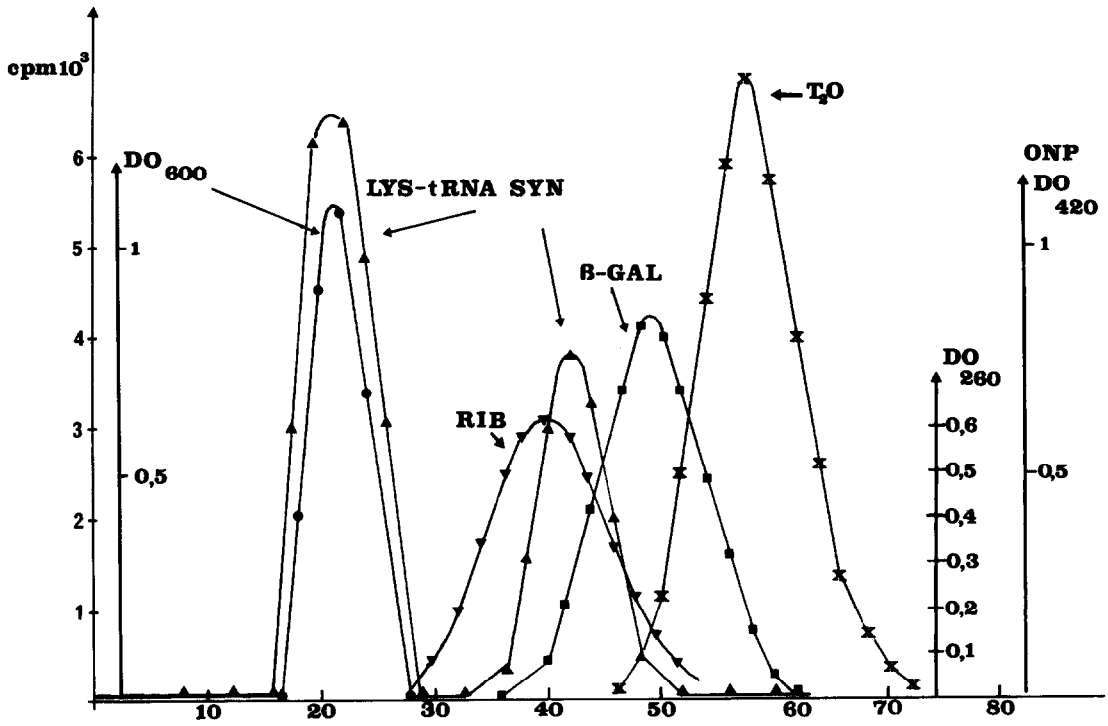

Fig.2. Fractionation of activity of the lysyl-tRNA synthetase by a column of 150 M agarose. A 2 × 14.5 cm column is equilibrated with the buffer: 0.05 M Tris–HCl (pH 7.6), 1 mM EDTA, 3 mM $MgCl_2$, 0.5 M dithiothreitol and 0.2 mM PMSF. 2 ml of enzyme extract and 20 μl of β-galactosidase are placed on the column. The column is then eluted with the same buffer solution. 0.7 ml fractions are collected and assayed for the formation of lysyl-tRNA and for the activity of the β-galactosidase. Next, 1.5 mg of yeast ribosomes and 100 μl of tritiated water (T_2O) (1.13×10^6 dpm/ml) are placed on the column and are eluted under identical conditions. (▲ ▲ ▲) Lysine tRNA synthetase. (■ ■ ■) β-Galactosidase. (× × ×) Tritiated water. (● ● ●) Cytoplasmic membranes. (▼ ▼ ▼) Ribosomes.

aminoacyl-tRNA synthetases in a cellular complex, with the tRNA and the elongation factors [7] supports the hypothesis that the in vivo protein synthesis apparatus consists of highly organized structures within eucaryotic cells, which are probably carried on the cytoplasmic membranes [18]. Indeed, the relatively small efficiency of the protein synthesis system in vitro as compared to synthesis in vivo may result from the disorganization of these structures.

Acknowledgements

We would like to thank Professor Claude Burstein for his valuable advice throughout our work and Mr Richard Buckingham for helping us with the writing of this article.

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